

## Claims:

1. A novel mycelial strain of edible mushroom *Termitomyces clypeatus* having enhanced cellobiase activity useful for better hydrolysis of cellulose, which has been deposited in Indian Institute of Chemical Biology, Calcutta with accession number IICB-411 and being deposited at ATCC having accession No.....
2. A novel strain as claimed in claim 1, wherein the mycelial culture of the edible mushroom *Termitomyces clypeatus* strain having the following physical ( i to vii) and bio chemical (viii to xiii ) characters;
  - i) the strain produces silky white colony on solid medium and frequently producing aggregation of mycelia in areas to form strands and knots of mycelia,
  - ii) the hyphae produced are tubular undifferentiated and hyphae of subculits with short branch are also present,
  - iii) the average diameter of the hyphae is between 5 to 10 micrometer and all are septed,
  - iv) the strain under microscope shows absence of any sporophoric structure or any basidiospore,
  - v) a few clamp connections characteristic of mushroom culture is also visible within the hyphae. Hyphae with both single and double nucleus are present,
  - vi) the culture remain white over a long period of growth, say 10 to 15 days without any coloration due to any sporulation,
  - vii) the mycelial culture is a brown-rot type and has no phenol oxidase or laccase activity,
  - viii) it grows well in defined medium containing carbohydrate and mineral salts,

- ix) the growth is not well supported by glucose but highly stimulated by glucose polysaccharides,
- x) the strain has two optimum C/N ratio at 5 and 10 for optimum growth in synthetic medium,
- xi) trace elements like  $\text{Fe}^{++}$ ,  $\text{Mn}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{Ca}^{++}$  and  $\text{Mo}^{++}$  have much influence on growth,
- xii) the dry mycelia obtained in liquid medium at optimum growth phase has composition as : protein 31.76 %, carbohydrate 52.0%, fat 1.0%, fiber 10.5% and ash 2.7%, and
- xiii) the strain in suitable medium gives positive tests for Carboxymethyl cellulase, Cellobiase. Invertase, Amylglucosidase, amylase, endo-xylanase, arabinofuranosidase, acety esterase and xylosidase activities but negative tests for laccase, phenol oxidase, chitinase and mannanase.
3. A method for enhancing the cellobiase activity of strain *Termitomyces clypeatus*, using 2-deoxy-D-glucose as glycosylation inhibitor, said method comprising steps of;
- (a) obtaining a culture medium of (the edible mushroom) *Termitomyces clypeatus*, (having an accession number IICB-411 given by Indian Institute of Chemical Biology, Calcutta, constituent laboratory of the applicants being deposited at ATCC and will be given reference no ..... ) by inoculating and growing mycelial culture of *Termitomyces clypeatus* in sterilized medium containing 0.05 to 5.0 mg/ml of 2-deoxy-D-glucose at pH between 3 to 8 and incubating at temperatures between 20-37°C under shaking in aerobic conditions,
- (b) separating the culture medium by known methods, and
- (c) using the culture filtrate directly as the source of the enzyme cellobiase and also for endo-glucanase and cellobiohydrolase for use in cellulose hydrolysis.
4. A process as claimed in claim 3, wherein the mycelial culture of the edible mushroom *Termitomyces clypeatus* strain has the following characters;

Physical properties: The strain produces silky white colony on solid medium and frequently producing aggregation of mycelia in areas to form strands and knots of mycelia. The hyphae produced are tubular undifferentiated and hyphae of subunits with short branch are also present. The average diameter of the hyphae is between 5 to 10 micrometer and all are septed. The strain under microscope shows absence of any sporophoric structure or any basidiospore. A few clamp connections characteristic of mushroom culture is also visible within the hyphae. Hyphae with both single and double nucleus are present. The culture remain white over a long period of growth, say 10 to 15 days without any coloration due to any sporulation.

Biochemical properties: The mycelial culture is a brown-rot type and has no phenol oxidase or laccase activity. It grows well in defined medium containing carbohydrate and mineral salts. The growth is not well supported by glucose but highly stimulated by glucose polysaccharides. The strain has two optimum C/N ratio at 5 and 10 for optimum growth in synthetic medium. Trace elements like  $\text{Fe}^{++}$ ,  $\text{Mn}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{Ca}^{++}$  and  $\text{Mo}^{++}$  have much influence on growth. The dry mycelia obtained in liquid medium at optimum growth phase has composition as : protein 31.76 %, carbohydrate 52.0%, fat 1.0%, fiber 10.5% and ash 2.7%. The strain in suitable medium gives positive tests for Carboxymethyl cellulase, Cellobiase. Invertase, Amylglucosidase, amylase, endo-xylanase, arabinofuranosidase, acety esterase and xylosidase activities but negative tests for laccase, phenol oxidase, chitinase and mannanase.

5. A process as claimed in claim 3 wherein, the 2-deoxy-D-glucose is used in the range between 0.05 to 5.0 %.
6. A process as claimed in claim 3 wherein, the strain is cultivated on the medium containing assimilable carbon and nitrogen sources, inorganic salts and organic nutrients, in presence of glycosylation inhibitors.
7. A process as claimed in claim 3 wherein, the assimilable carbon sources used are carbohydrates selected from cellobiose, mannose, fructose, xylose, arabinose, starch, dextrin, cellulose, cotton, xylan and/or agrowastes selected from baggasse powder, rice-

straw powder, wheat bran, corn cob powder, corn powder in presence of TCA cycle acids selected from succinate, fumarate, maleate or amino acids selected from aspartate, glutamate, serine, histidine and alanine or glucose analogue D-glucosamine.

8. A process as claimed in claim 3 wherein, the glycosylation inhibitors is selected from tunicamycin, deoxy nojirimycin, 2-deoxy-D-glucose and D-glucono-lactone.
9. A process as claimed in claim 3 wherein, the assimilable nitrogen sources used are selected from ammonium chloride, ammonium nitrate, ammonium di hydrogen orthophosphate, and potassium nitrate.
10. A process as claimed in claim 13 wherein, the organic nutrients used are selected from malt extract, yeast extract, potato extract, peptone, soya-peptone, bactopectone, and corn steep liquor.
11. A process as claimed in claim 3 wherein, detergents used are selected from Tween-20, Tween-80, and Tween-100.
12. A process as claimed in claim 3 wherein, the strain *Termitomyces clypeatus* also provides endo-glucanase activity and cellobiohydrolase activity for hydrolysis of cellulose to glucose.